ratio between the intensities of the first and second fluorescent signals at a given time.

Claim 13.

In addition, neither reference teaches or suggests a method which includes the step of

calculating a corrected intensity signal corresponding to a ratio between the intensity of the first and second fluorescent signals at a given time before and after amplifying the nucleic acid sequence, a change in the corrected intensity signal after amplification indicating the formation of the nucleic acid amplification reaction product.

Claim 39.

While Higuchi, et al. teaches a fiber optic device for monitoring PCR reactions, Higuchi, et al. does not teach or suggest the problem identified by Applicants that small variations in base line fluorescence due to system based variations exist when monitoring a sample over time and thus does not teach or suggest a mechanism for correcting this error (detection and analysis mechanism) or a way of operating an apparatus to correct for this error.

As the Examiner indicates, Lee, et al. teaches that TMR can be used as an internal standard for pipetting errors and evaporation. However, pipetting errors are a constant error introduced when the sample is prepared. Only a single measurement need be taken in order to determine and correct for this error. Evaporation can be addressed by sealing the reaction vessel, or by taking initial and final concentration measurements since evaporation can be assumed to occur substantially linearly over time.

As Applicants show in Figure 4, fluorescence can vary cycle to cycle due to system based variations which are independent of pipetting errors or evaporation. For example, since no reagents were added between cycles, the variations shown in Figure 4 are clearly not due to a pipetting error. Given that the sample was sealed in this example, the observed variations are also not due to evaporation. Further, as can be seen from the TAMARA plot, system based variations can increase and decrease over time. By contrast, evaporation can only result in an increase in fluorescence over time due to an increase in sample concentration. Accordingly, Lee's teaching regarding the use of an internal standard to compensate for pipetting errors and evaporation does not solve the same problem being

solved by the present invention and thus cannot be interpreted as teaching or suggesting a need to calculate corrected intensity signals as they are currently being claimed.

Lee, et al. also cannot be interpreted as teaching, suggesting or motivating the particular method for using an internal standard as it is being claimed in the pending apparatus and method claims. Rather, the importance of calculating corrected intensity signals in the manner claimed is only taught in the present application with regard to eliminating system based errors in order to reduce the RMS of fluctuations in the readout signal to less than 1% of the average magnitude of the measured ratio. Specification, page 14, lines 8-12, Figure 5. As discussed above, one does not need to use an internal standard in the manner claimed in order to address the sources of error identified by Lee, et al.

Even if the combination of Higuchi, et al. and Lee, et al. were to set forth a *prima facie* case for obviousness, the particular system based variations identified in the present application and Applicants' efficient elimination of these previously unidentified variations using the claimed apparatus and method represents an unexpected result which rebut any *prima facie* case.

Since Applicants are the first to recognize the problem of system based fluorescence variations in a single sample when monitoring the formation of a nucleic acid amplification reaction product in real time and the solution thereto, Applicants are entitled to a patent to their solution to this problem. The Examiner is therefore respectfully requested to withdraw the present rejection for obviousness in view of this distinction over the prior art.

Applicants earnestly believe that they are entitled to a letters patent, and respectfully solicit the Examiner to expedite prosecution of this patent application to issuance. Should the Examiner have any questions, the Examiner is encouraged to telephone the undersigned.

Respectfully submitted,

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